Importance of mass and light levels for germination of achenes of *Coreopsis tinctoria* Nutt. (coreopsis, goldenwave, Asteraceae)

K. C. Eddy
Department of Civil and Environmental Engineering
The University of Texas at San Antonio, San Antonio, TX 78249
kevinceddy@gmail.com

O. W. Van Auken
Department of Biology, The University of Texas at San Antonio, San Antonio, TX 78249
oscar.vanauken@utsa.edu

ABSTRACT

Coreopsis tinctoria Nutt. (coreopsis, goldenwave, Asteraceae) is a spring and early summer flowering annual found throughout most of North America. Its apparent habitat is primarily vegetation gaps or patches in disturbed grasslands and woodlands. It was hypothesized that achene mass and light levels were important for achene germination. Achene mass, determined by seed buoyancy, was used as a surrogate to determine viability. All collected achenes were presumed viable. To test the effectiveness of achene mass as a surrogate for determining seed viability, mean germination of high mass (non-buoyant) achenes was compared to mean germination of low mass (buoyant) achenes. The role of light level was investigated by germinating collected achenes at three light levels: 121.50, 0.01, and 0.00 $\mu mol \cdot m^{-2} s^{-1}$. The float test correctly identified viable achenes 80% of the time. There were significantly fewer germinations (5±2, mean±standard deviation, 20%) of the low mass achenes than for the high mass achenes (21±4, 84%), indicating that achene mass was a fast and easy surrogate for determining *C. tinctoria* achene viability. The mean germination of achenes in the light treatments was 84% (21±4) with no significant differences in the mean number of achenes that germinated in the three light treatments. In addition, 56% of the achenes germinated four days after placement into a moist environment and 84% germinated in eight days, suggesting a lack of dormancy. Published on-line **www.phytologia.org** *Phytologia 96(3): 159-166 (July 1, 2014).* ISSN 030319430

KEY WORDS: achene mass, seed buoyancy, float test, seed germination, achene germination, *Coreopsis tinctoria*, coreopsis, goldenwave, plains coreopsis, calliopsis, golden tickseed, seed/achene viability

---

Successful establishment of a plant from seed requires favorable environmental conditions for seed germination (Fenner 1985). Typical environmental conditions required for germination include appropriate temperature, soil moisture, and oxygen levels. In some cases, light level, photoperiod, and the red/far-red light ratios are also necessary for germination (Baskin and Baskin 1998). Factors that control seed germination can give insights into the habitat and growth requirements of a species (Van Auken 2013). Seed mass, measured by buoyancy in water, is a common, non-destructive, rapid technique used to estimate the viability of collected seeds (Paynter and Dixon 1990, Gribko and Jones 1995, Dalgleish et al. 2012). However, its use has not been tested with *Coreopsis tinctoria* achenes (seeds). Many non-dormant seeds need light for germination, and it has been reported that *C. tinctoria* achenes do require certain light levels for germination (Baskin and Baskin 1998).

Coreopsis tinctoria (Fig. 1) is commonly known as goldenwave, plains coreopsis, calliopsis, or golden tickseed (Native Plant Information Network 2009). It is an early spring and early summer flowering annual in the Asteraceae family that reaches heights between 250 and 1200 cm (Correll and Johnston 1979, Native Plant Information Network 2009), and is widely distributed throughout most of the

lower 48 United States, parts of southern Canada, and northern Mexico (Fig. 2) (Strother 2006, USDA 2012). It can tolerate various soil types, but seems to prefer moist sandy or clayey soils (Enquist 1987, Strother 2006). It is not a good competitor (Elliott and Van Auken 2014) similar to other species of *Coreopsis* (Folgate and Scheiner 1992) and appears to grow best in open grassland patches, disturbances, or gaps. It was hypothesized that achene mass and light levels were important for achene germination in *C. tinctoria*.

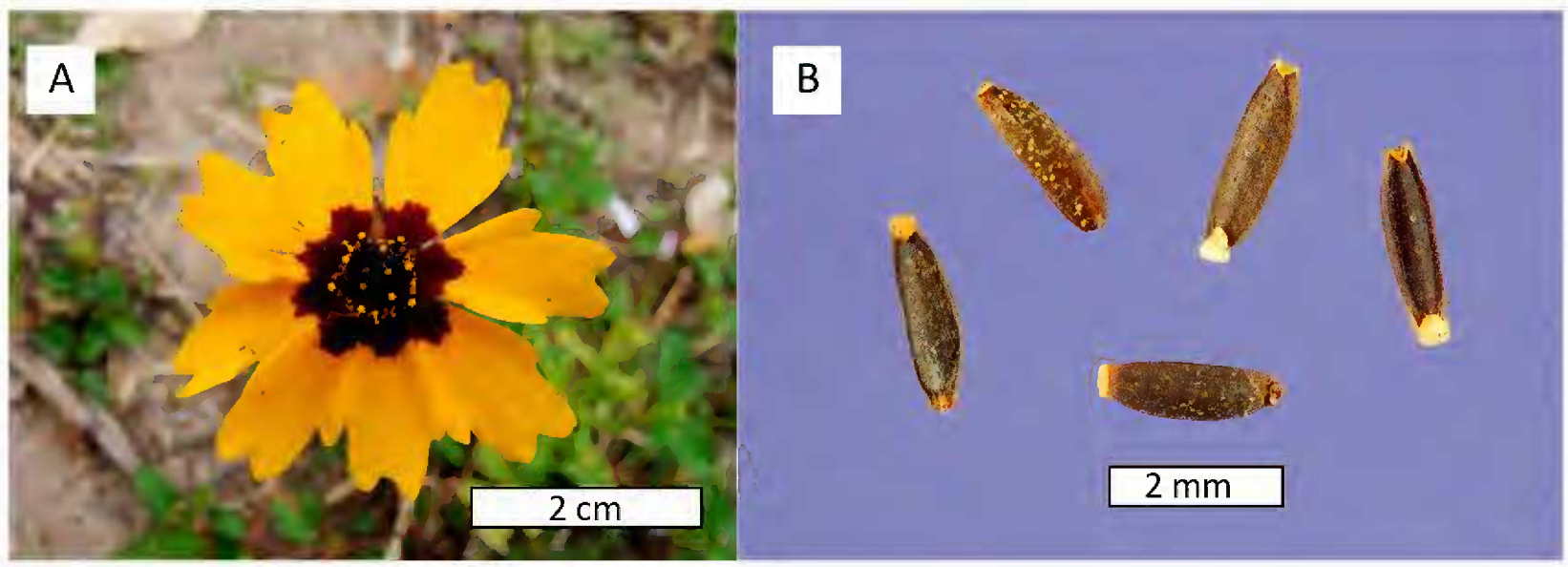

Figure 1. *Coreopsis tinctoria* flower head (A – Photograph by K. C. Eddy, senior author) and achenes (B – Photograph by Steve Hurst (USDA 2012)).

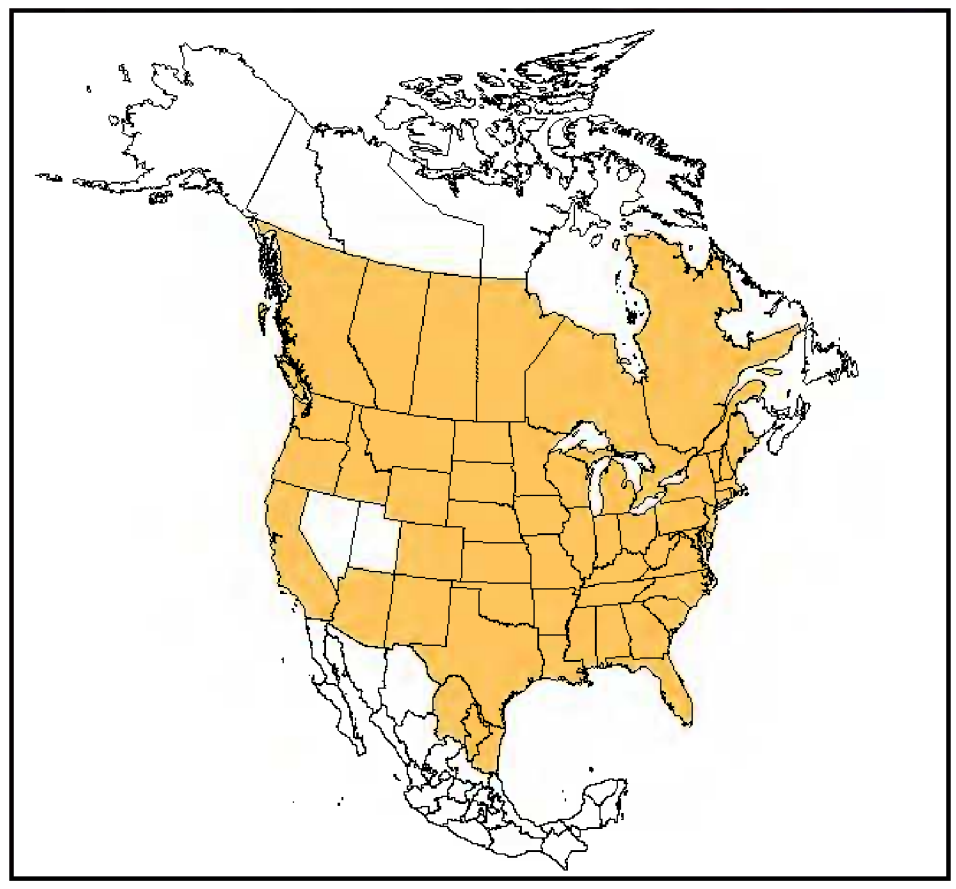

Figure 2. Distribution (shaded area) of *Coreopsis tinctoria* in the United States, Canada, and northern Mexico (Strother 2006). Albers equal-area conic projection.

MATERIALS AND METHODS

Mature *Coreopsis tinctoria* seed heads were harvested from various locations in northwestern Bexar County, Texas (29°35' N, 98°37' W), in May of 2011. Seed heads were dried and stored at room temperature in paper sacks for six months prior to starting the experiments. Seed heads were then crushed in a resealable plastic storage bag and sieved with a 2 mm sieve to remove most chaff from the achenes (seeds). Achenes were then removed from remaining chaff by picking with tweezers or a metal spatula.

Achene mass (buoyancy) (Arrillaga et al. 1992, Elliott 1999) was tested as a potential surrogate for viability of collected achenes. One half ml of detergent was added to 400 ml deionized water to reduce surface tension. Thirty-five batches of 30 achenes each were used to separate low mass and high mass achenes in the water-detergent solution. Following separation of the low mass and high mass achenes, they were rinsed in deionized water, dried, and then a portion of each group was tested for ability to germinate.

The germination test consisted of two treatments (low mass and high mass achenes), each with eight replicates. Each replicate consisted of twenty five achenes placed on a 700 mm diameter Whatman® #4 qualitative filter paper in a petri plate. One ml of deionized water was placed on the filter paper; the plates were covered, placed in resealable plastic storage bags to prevent evaporation, and placed in the laboratory at low light and at approximately 25° C. Throughout the experiment, 1 ml of deionized water was added every two days, if needed, to keep the filter paper moist. Achenes were checked for germination every other day for two weeks. Germination occurred when the radicle emerged and was >1 mm long (Baskin and Baskin 1998, Elliott 1999). Achenes that germinated were counted and removed from the petri plates. The plates were then replaced in the plastic storage bags which were resealed. The number of achenes that germinated and the length of time required for germination were recorded. The mean total number of achenes that germinated was compared using a Wilcoxon rank-sum test. The number that germinated in time was compared using a Kruskal-Wallis one-way non-parametric ANOVA followed by pairwise comparisons using the Wilcoxon rank-sum test (Sall et al. 2012).

Achene germination was also determined at three light levels. Light levels were 121.50 $\mu mol \cdot m^{-2} s^{-1}$, 0.01 $\mu mol \cdot m^{-2} s^{-1}$, and 0.00 $\mu mol \cdot m^{-2} s^{-1}$. Light levels were measured using a Li-Cor® LI-190 Quantum Sensor attached to a Li-Cor® LI-1000 Data Logger. Temperatures were approximately 25^0 C. Every other day, for two weeks, the achenes were checked for germination. Germination occurred when the radicle emerged and was >1 mm long (Baskin and Baskin 1998, Elliott 1999). Those that germinated were counted and removed from the petri plates and the plate was replaced in the treatment as above. The number of achenes that germinated and the time required for germination were recorded. The mean number of germinations in the light treatments was compared using a one-way non-parametric ANOVA and pairwise comparisons, as above (Sall et al. 2012).

Because there were no significant differences in the number of germinations between the light treatments, results from these three treatments were pooled to compare germinations through time. The number of achenes that germinated per two days was plotted as was the sum of the mean number of achenes that germinated. In addition, the mean and standard deviation of the total number of achenes that germinated in each treatment was plotted as a bar graph.

RESULTS

In the achene mass experiment, 52% of all collected achenes germinated; 20% (5±2, $\bar{x} \pm$ SD) of the low mass achenes, which was significantly fewer than the 84% (21±4) of high mass achenes (Fig. 3, Wilcoxon rank-sum, H=11.41, P=0.0007). On day 4, 3±2 low mass achenes germinated, significantly more than on any other day (Fig. 4; one-way non-parametric ANOVA, H=25.67, P<0.0001, Kruskal-Wallis followed by Wilcoxon rank-sum). Significantly more high mass achenes germinated on day 4 (18±3) than on any other day, and significantly more high mass achenes germinated on day 6 (3±3) than on days 2, 8, and 10 (Fig. 4; one-way non-parametric ANOVA, H=33.40, P<0.0001, Kruskal-Wallis followed by Wilcoxon rank-sum). There were no further low mass or high mass achene germinations after day 6 (Figs. 4 & 5).

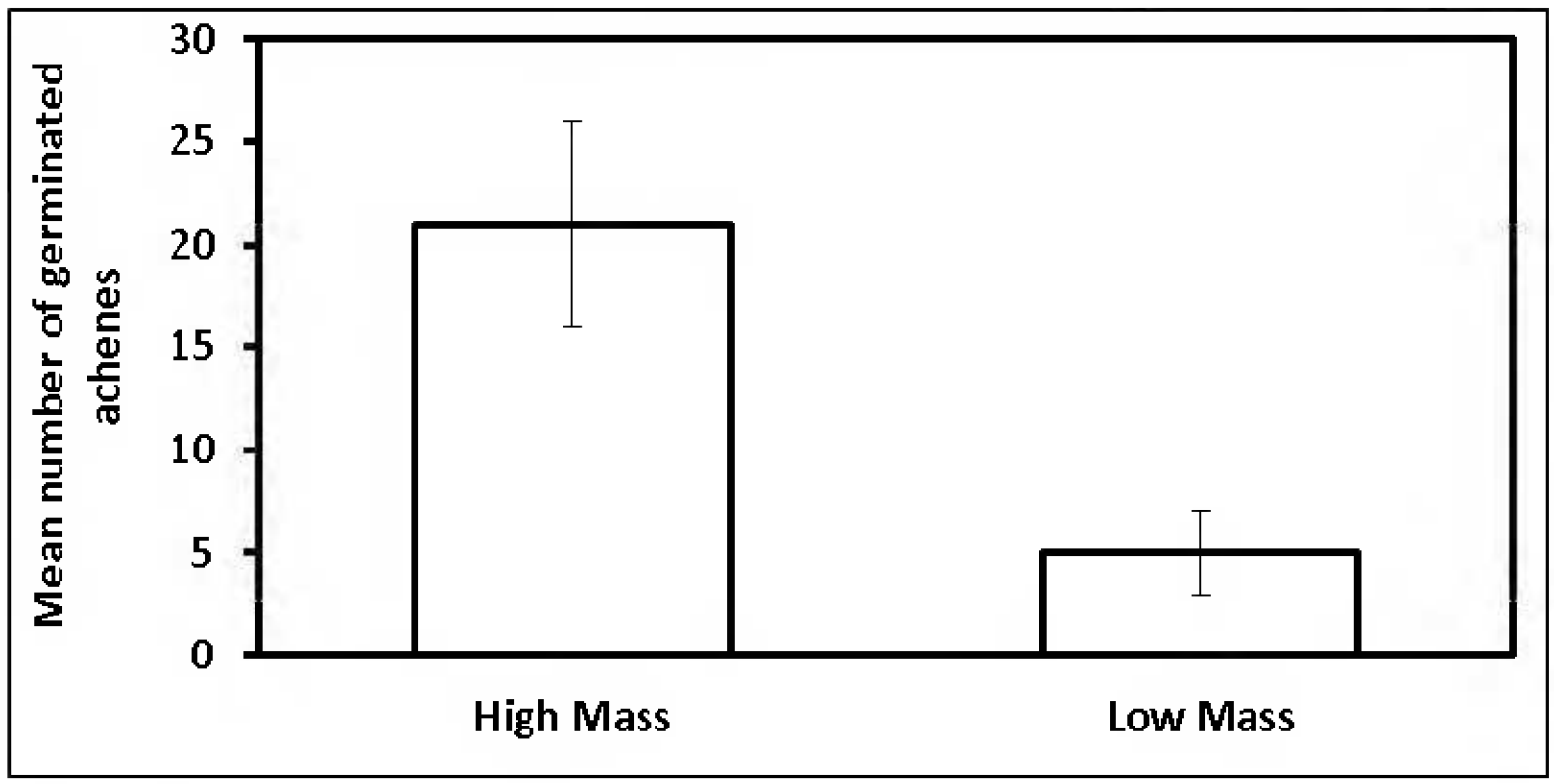

Figure 3. Comparison of the mean total number of germinated achenes after separation by mass. Of the high mass achenes, 84% germinated (21±4), significantly more than the 20% of low mass achenes that germinated (5±2) (Wilcoxon rank-sum one-way non-parametric ANOVA, H=11.41, P=0.0007). Error bars indicate ± one standard deviation of the mean.

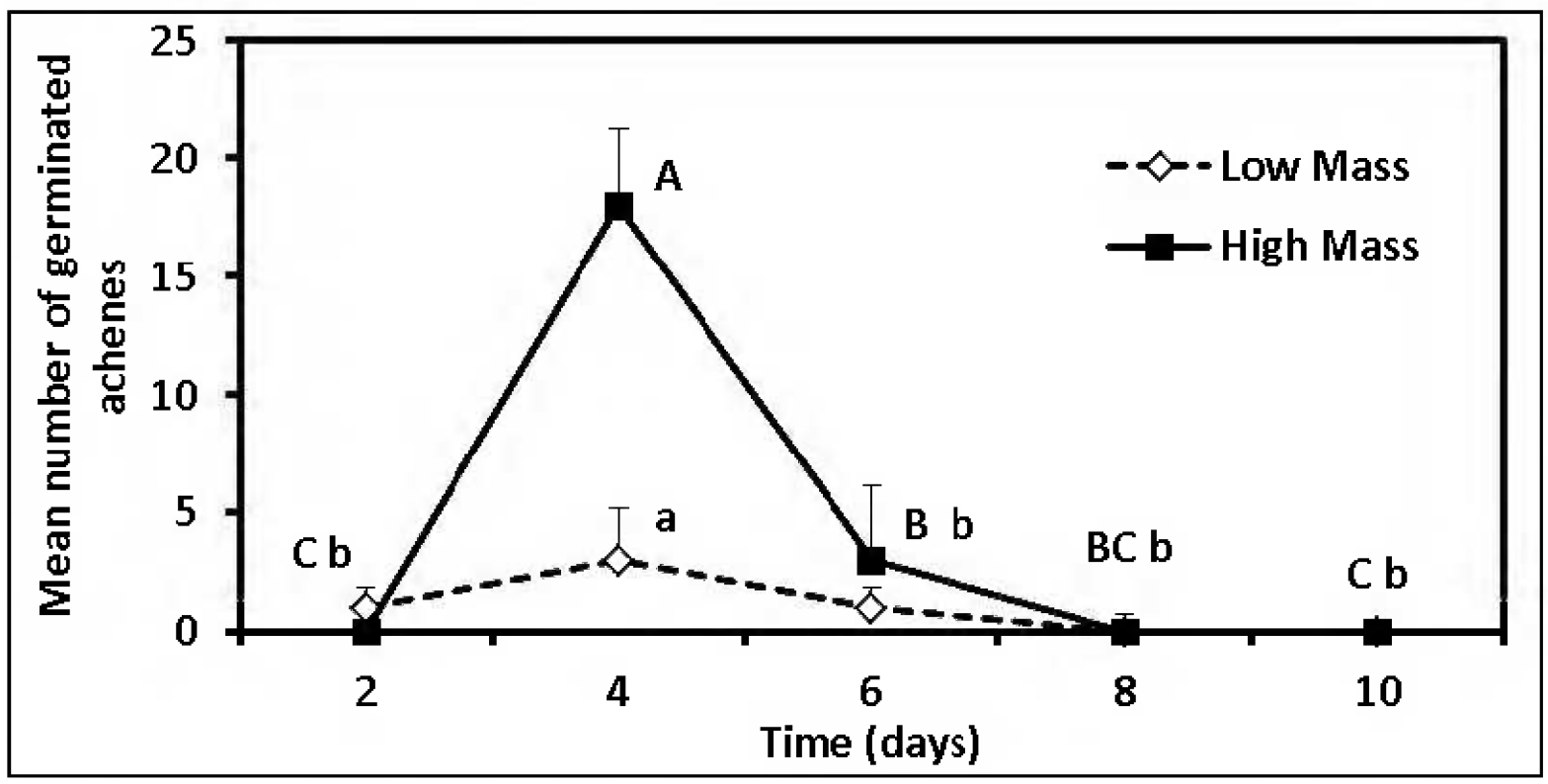

Figure 4. Comparison of low mass and high mass achene germinations per two days (error bars indicate + one standard deviation of the mean). Significantly more low mass or high mass achenes germinated on day 4 than on any other day (Kruskal-Wallis one-way non-parametric ANOVA, H=25.67, P<0.0001 and H=33.40, P<0.0001, respectively, Wilcoxon rank-sum pairwise comparison). For either type of achene, the same upper case (high mass) or lower case (low mass) letters indicate no significant difference.

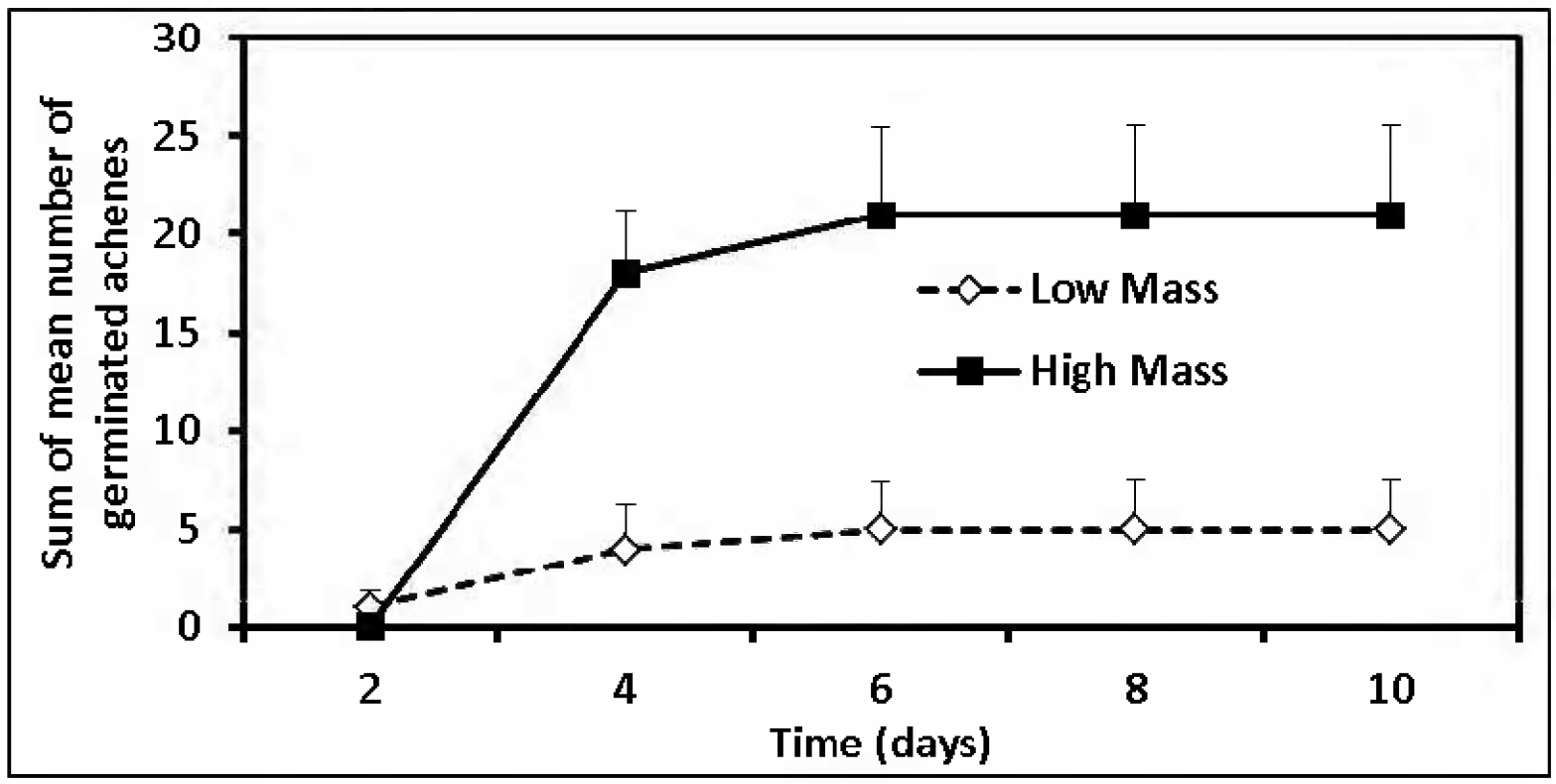

Figure 5. Comparison of the sum of the mean of low mass and high mass achene germinations per two days (error bars indicate + one standard deviation of the mean). Out of a total of 25 seeds used in each replicate, 5±2 low mass achenes germinated and 21±4 high mass achenes germinated. In both cases, there were no further germinations after day six.

In the light treatment experiment, the mean number of germinated achenes at 125.50 µmol m^{-2} s^{-1} (21±4) was not significantly different than the 20±2 achenes germinated at 0.01 µmol m^{-2} s^{-1} or the 20±3 achenes germinated at 0.00 µmol m^{-2} s^{-1} (Fig. 6, one-way nonparametric ANOVA, H=1.77, P=0.4121, Kruskal-Wallis). Examination of the pooled results shows that significantly more achenes germinated on day 4 (14±4) than any other day, and significantly more germinated on day 6 (6±4) than days 2, 8, or 10 (Fig. 7, one-way non-parametric ANOVA, H=100.91, P<0.0001, Kruskal-Wallis followed by Wilcoxon rank-sum). No more achenes germinated after day 8 (Fig. 8). Overall, approximately 84% of all tested achenes in the light treatments germinated or conversely 16% did not germinate and appeared nonviable.

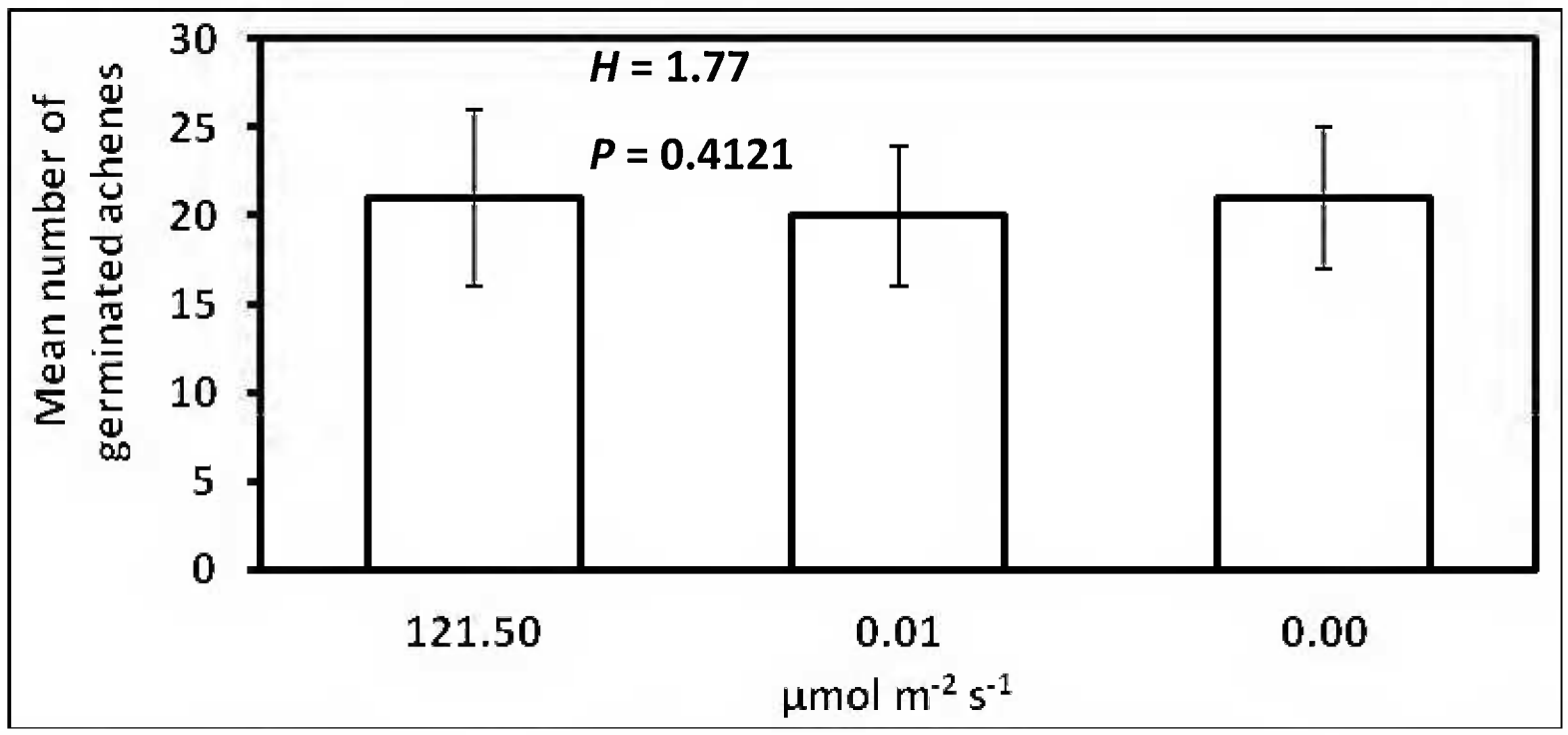

Figure 6. Comparison of the mean number of germinated achenes in each light treatment. There were no significant differences in mean number of germinations between light treatments (Kruskal-Wallis one-way non-parametric ANOVA, H=1.77, P=0.4121). Error bars indicate ± one standard deviation of the mean.

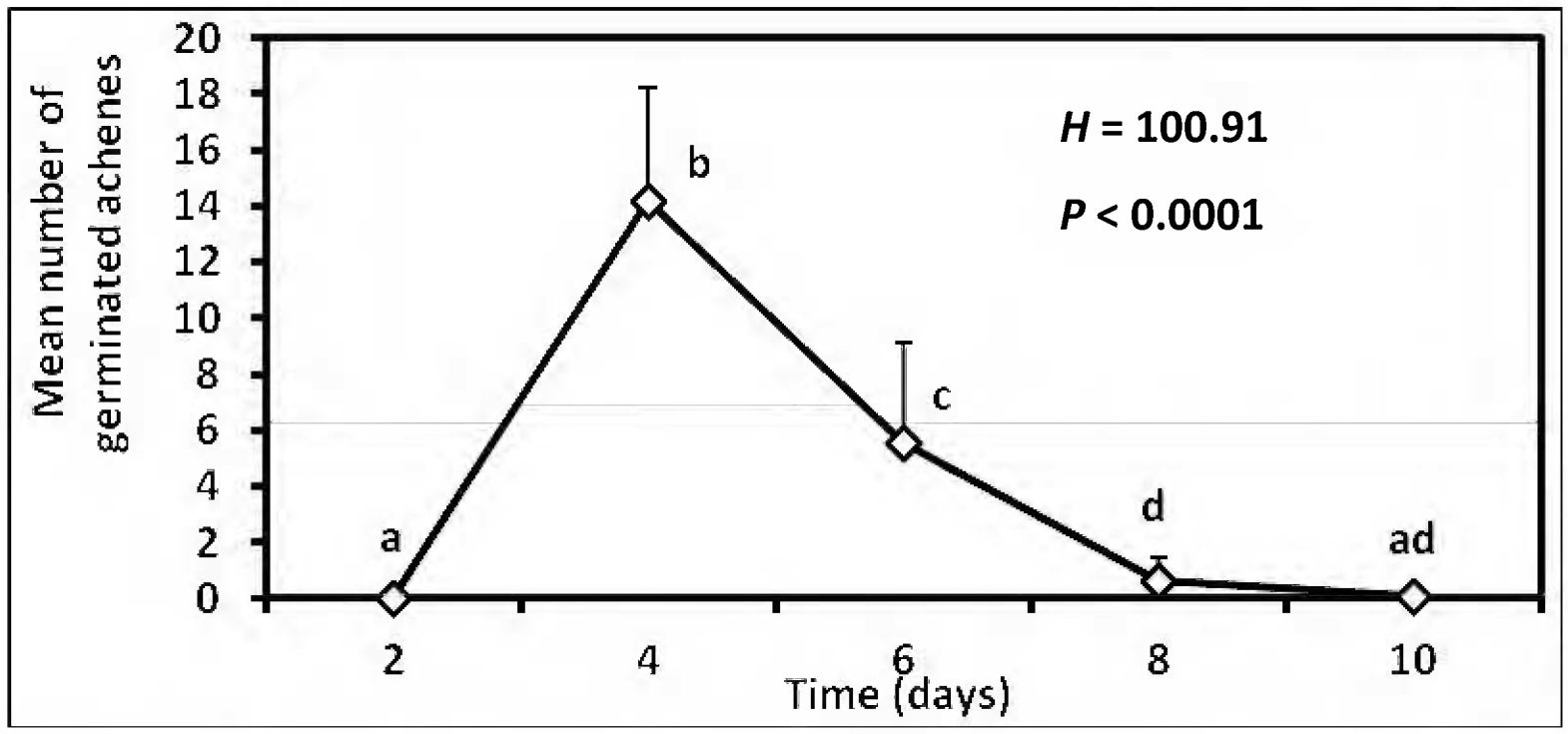

Figure 7. Pooled mean number of germinated achenes in the light treatments per two days. Sixty six percent of the achenes germinated through day 4 (14±4), significantly more than any other day (Kruskal-Wallis one-way non-parametric ANOVA, H=100.91, P<0.0001, Wilcoxon rank-sum pairwise comparison). Different letters indicate a significant difference. Error bars indicate + one standard deviation of the mean.

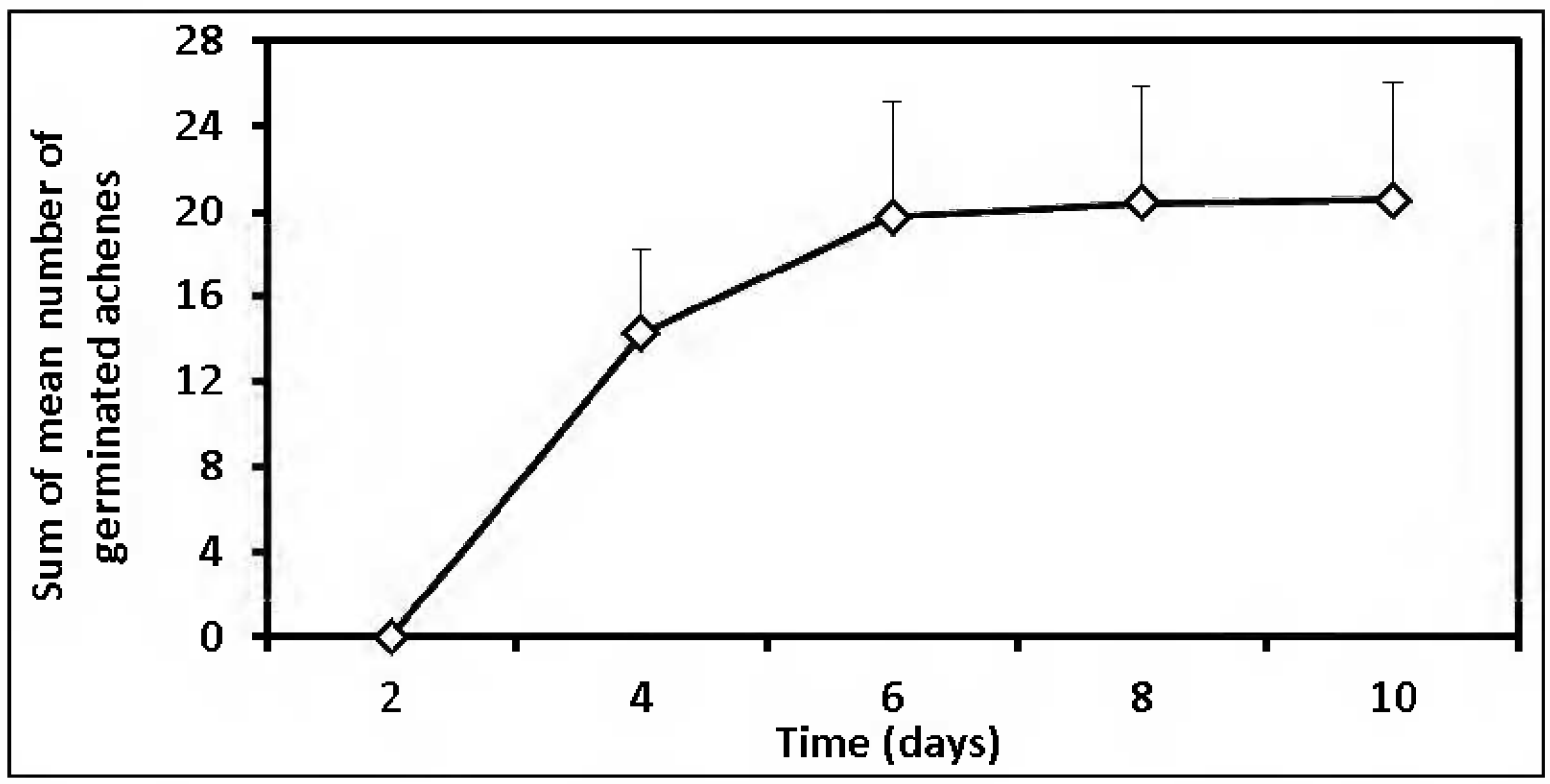

Figure 8. Pooled sum of mean number of achenes germinated per two days. After 8 days, 21±4 achenes had germinated. There were no further germinations after 8 days. Error bars indicate + one standard deviation of the mean.

DISCUSSION

For a plant to be successful, its seeds must germinate when environmental conditions favor the survival of seedlings and are conducive to their growth and reproduction (Fenner 1985, Kettenring et al. 2006). By understanding the germination requirements of seeds, seedlings could be available for experimentalists to do ecological, physiological, or genetic studies and to better understand species interactions in the environment, including competition (Smith and Smith 2012). Interspecific competition has been shown to negatively affect the growth of *C. tinctoria* (Eddy 2013, Elliott and Van Auken 2014)

and *C. lanceolata* (Folgate and Scheiner 1992) and determine where *C. tinctoria* occurs by limiting it to physical or temporal gaps (Eddy 2013, Elliott and Van Auken 2014).

Of all collected achenes, 52% germinated, comparable to 48 – 80% for *C. lanceolata* (Norcini and Aldrich 2007), and 69% for *C. leavenworthii* (Kabat et al. 2007). Buoyancy, or the float test, commonly used in horticulture to distinguish viable and nonviable seeds or achenes (Czarnota et al. 2003), appears to be a reasonable, fast, and simple method for determining viability of *C. tinctoria* achenes, with only 20% of the viable achenes misidentified as nonviable. Seed morphology and predation may be factors that affect the usefulness of flotation testing for estimating seed viability. Comparing floatation testing and seed x-raying as viability estimation techniques, the float test misidentified chestnuts damaged by seed predation 50% of the time (Dalgleish et al. 2012). However, for acorns the test was correct 91.6% of the time (Gribko and Jones 1995). Flotation testing may not be appropriate for plants that would use buoyancy as a method of seed dispersal, as is the case with some wetland plants (van den Broek et al. 2005).

Light levels and photoperiod have been shown to be important for germination in various members of the family Asteraceae (Meyer et al. 1990, Baskin and Baskin 1998, Elliott 1999). However, in the current study variation in low light levels was not a factor in *C. tinctoria* germination. In a study of *C. leavenworthii* that incorporated photoperiod, exposure to light significantly increased achene germination, while buried achenes did not germinate but became part of the seed bank, suggesting they would have to be brought to the surface by some disturbance before they would germinate (Kabat et al. 2007). The apparent lack of a light effect combined with rapid germination (most achenes germinated by day 4, Fig. 6) suggests that *C. tinctoria* achenes are not dormant. This may allow *C. tinctoria* to germinate as soon as environmental temperature and soil moisture conditions become favorable for growth, giving *C. tinctoria* a growth advantage in gaps or patches in C_4 grasslands where competition for resources may be very intense (Elliott 1999, Smith and Smith 2012, Eddy 2013). A light requirement has been reported for *C. tinctoria* germination (Baskin and Baskin 1998), but not in this experiment, thus further research is needed to clarify the role of light levels in *C. tinctoria* germination.

ACKNOWLEDGEMENTS

The authors would like to thank the manuscript reviewers, Karen Engates, Wendy Leonard, Fernando Martinez, and Heather Shipley, for their many helpful comments and suggestions.

LITERATURE CITED

Arrillaga, I., T. Marzo, and J. Segura. 1992. Embryo culture of *Fraxinus ornus* and *Sorbus domestica* removes seed dormancy. Hortscience **27**:371-371.

Baskin, C. C. and J. M. Baskin. 1998. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, New York.

Correll, D. S. and M. C. Johnston. 1979. Manual of the vascular plants of Texas. The University of Texas at Dallas, Richardson, Texas.

Czarnota, M. A., A. M. Rimando, and L. A. Weston. 2003. Evaluation of root exudates of seven sorghum accessions. Journal of Chemical Ecology **29**:2073-2083.

Dalgleish, H. J., J. T. Shukle, and R. K. Swihart. 2012. Weevil seed damage reduces germination and seedling growth of hybrid American chestnut. Canadian Journal of Forest Research **42**:1107-1114.

Eddy, K. C. 2013. Are temporal gaps necessary for the establishment of the native wildflower, *Coreopsis tinctoria*? MS Thesis. University of Texas at San Antonio, San Antonio, Texas.

Elliott, S. A. 1999. *Coreopsis tinctoria*: Germination requirements and competitive ability with a perennial grass. MS Thesis. The University of Texas at San Antonio, San Antonio, Texas.

Elliott, S. A. and O. W. Van Auken. 2014. Competition and niche requirements of *Coreopsis tinctoria* a widespread but locally abundant annual Asteraceae. Submitted to Madroño.
Enquist, M. 1987. Wildflowers of the Texas Hill Country. Lone Star Botanical, Austin, Texas.
Fenner, M. 1985. Seed ecology. Chapman and Hall, New York.
Folgate, L. A. and S. M. Scheiner. 1992. Distribution of a restricted locally abundant species: Effects of competition and nutrients on *Coreopsis lanceolata*. American Midland Naturalist **128**:254-269.
Gribko, L. S. and W. E. Jones. 1995. Test of the float method of assessing northern red oak acorn condition. Tree Planters Notes **46**:143-147.
Kabat, S. M., B. Dehgan, and J. G. Norcini. 2007. Temperature and light affects germination ecology of commercially produced seeds of Leavenworth's coreopsis. Native Plants Journal **8**:236-247.
Kettenring, K. M., G. Gardner, and S. M. Galatowitsch. 2006. Effect of light on seed germination of eight wetland *Carex* species. Annals of Botany **98**:869-874.
Meyer, S. E., S. B. Monsen, and E. D. McArthur. 1990. Germination response of *Artemisia tridentata* (Asteraceae) to light and chill: Patterns of between-population variation. Botanical Gazette **151**:176-183.
Native Plant Information Network. 2009. *Coreopsis tinctoria*. Lady Bird Johnson Wildflower Center at The University of Texas, Austin, Texas.
Norcini, J. G. and J. H. Aldrich. 2007. Storage effects on dormancy and germination of native tickseed species. HortTechnology **17**:505.
Paynter, B. H. and K. W. Dixon. 1990. Seed viability and embryo decline in *Geleznowia verrucosa* Turcz. (Rutaceae). Scientia Horticulturae **45**:149-157.
Sall, J. A., A. Lehman, M. Stephens, and L. Creighton. 2012. JMP start statistics: a guide to statistics and data analysis using JMP. SAS Institute, Inc., Cary, NC.
Smith, T. M. and R. L. Smith. 2012. Elements of ecology. 8th edition. Pearson Benjamin Cummings, San Francisco.
Strother, J. L. 2006. *Coreopsis tinctoria*. Pages 185, 194, 197-198 *in* Flora of North America Editorial Committee, editor. Flora of North America North of Mexico, Vol 21: Asteraceae. Oxford University Press, New York.
USDA. 2012. The PLANTS database (plants.usda.gov, 4 November 2012). National Plant Data Team, Greensboro, North Carolina.
Van Auken, O. W. 2013. Germination of achenes of *Chaptalia texana* Greene (Silverpuff) a perennial Asteraceae. Phytologia **95**:255-263.
van den Broek, T., R. van Diggelen, and R. Bobbink. 2005. Variation in seed buoyancy of species in wetland ecosystems with different flooding dynamics. Journal of Vegetation Science **16**:579-586.